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PROSTATE-SPECIFIC MEMBRANE ANTIGEN EXPRESSION IS GREATEST IN PROSTATE ADENOCARCINOMA AND LYMPH NODE METASTASES

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ABSTRACT

Objectives. Prostate-specific membrane antigen (PSMA) is an integral membrane protein highly specific for the prostate. PSMA may be clinically useful for predicting outcome in patients with prostate cancer. We compared the expression of PSMA in prostate adenocarcinoma and lymph node metastases in a large series of patients with node-positive cancer.

Methods. We studied 232 patients with node-positive adenocarcinoma who underwent bilateral pelvic lymphadenectomy and radical retropubic prostatectomy at the Mayo Clinic between 1987 and 1992. Immunohistochemistry was performed using monoclonal antibody 7E11-5.3 directed against PSMA. For each case, the percentage of immunoreactive cells in benign prostate tissue, adenocarcinoma, and lymph node metastases was estimated in 10% increments. Intensity was recorded using a scale of 0 to 3 (0 = no staining, 3 = highest).

Results. Cytoplasmic immunoreactivity for PSMA was observed in all cases in benign epithelium and cancer, and most lymph node metastases. The number of cells stained was lowest in benign epithelium; cancer and lymph node metastases were similar (46.2% ± 27.5% versus 79.3% ± 18.5% versus 76.4% ± 26.1%, respectively; all pairs $P < 0.05$). Intensity of staining was greatest in primary cancer and lowest in lymph node metastases.

Conclusions. PSMA is expressed in benign prostatic epithelium and primary cancer in all cases and in 98% of cases with lymph node metastases. Expression of PSMA was greatest in primary cancer for both percentage and intensity of immunoreactive cells. PSMA expression allows the identification of benign and malignant prostatic epithelium and may be a potentially valuable marker in the treatment of patients with prostate cancer. UROLOGY 52: 637–640, 1998. © 1998, Elsevier Science Inc. All rights reserved.

Prostate cancer has an unknown etiology, variable pathology, and an intricate relationship with endocrine factors. It has the propensity for progression and dedifferentiation, which further adds to the complexity of this disease and limits effective therapies.[1] In 1998 prostate cancer will be diagnosed in an estimated 184,500 men, and 39,200 will die of this cancer.[2] The search for diagnostic and prognostic markers for cancer of the prostate is ongoing.

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Prostate-specific membrane antigen (PSMA) is a transmembrane glycoprotein with both intracellular and extracellular domains.[3,4] Its function appears to be as a cell-surface peptidase hydrolyzing peptides in prostatic fluid and generating glutamate[4,5] and also acts as a folate hydrolase.[6,7] It is expressed in benign and malignant prostatic epithelium and can be detected immunohistochemically. The antibody used in this study, 7E11-C5.3, recognizes the first six N-terminal amino acids of the cytoplasmic domain. Because PSMA is an integral membrane protein, it is being exploited as a target for antibody-directed imaging and therapeutic targeting modalities.[8–13]

In this report we describe the expression of PSMA in prostatic adenocarcinoma and lymph node metastases in a large series of patients with node-positive cancer.

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MATERIAL AND METHODS

PATIENTS

We studied select tissue sections from 232 previously untreated patients with prostate adenocarcinoma who underwent bilateral pelvic lymphadenectomy and radical retropubic prostatectomy at the Mayo Clinic between 1987 and 1992. Patients without sufficient cancer tissue available for analysis were excluded. The retrieval of tissue and processing have been previously described.[14,15] In brief, specimens were obtained during radical retropubic prostatectomy and pelvic lymphadenectomy. Each was evaluated by frozen section at the time of surgery and on permanent sections. Each prostate was weighed, measured, and inked. Shave margins were obtained from the apex and base. The remaining prostate was serially sectioned at 4 to 5-mm intervals perpendicular to the long axis of the gland from the apex to the tip of the seminal vesicles. All cases were fixed in neutral buffered formalin overnight and processed routinely to paraffin.

IMMUNOHISTOCHEMICAL STUDIES

Mouse monoclonal antibody 7E11-5.3 (Cytogen Corp., Princeton, NJ) directed against PSMA was used, as previously described.[15] The immunohistochemical technique included sequential application of diluted primary antibody (PSMA; 20 μg/ml) for 60 minutes, biotinylated goat anti-mouse immunoglobulin (Ig)G and goat anti-rabbit IgG (1:400, Dako Corp., Santa Barbara, Calif) for 30 minutes, and peroxidase-labeled streptavidin (1:500, Dako Corp.) for 30 minutes. Immunoreactivity was visualized by incubation of sections with 3-aminoethylcarbazole in the presence of hydrogen peroxide. Sections were counterstained with light hematoxylin and mounted with a coverslip. No enzyme pretreatment was used, and microwave antigen retrieval was not necessary (data not shown). Positive and negative controls were run in parallel with each batch and gave appropriate results.

The extent and intensity of staining for this antibody were evaluated in benign prostatic tissue, primary cancer, and lymph node metastases by two of the authors (D.G.B. and A.P.). The percentage of cells exhibiting staining in each case was estimated in 10% increments. Also, a numerical intensity score between 0 and 3 was assigned to each using the following criteria: 0 = no staining; 1 = weak equivocal staining; 2 = unequivocal moderate staining; 3 = strong staining. Only cells showing an intensity of staining greater than 1 were considered positive. In most cases, the staining was unequivocal.

STATISTICAL STUDIES

Spearman's rank correlations were used to compare the mean percentage of immunoreactive cells for benign epithelium, primary cancer, and lymph node metastases. The significance level was 0.05.

RESULTS

Cytoplasmic immunoreactivity for PSMA was noted in 100% of cases of benign epithelium and primary cancer and in 98% of lymph node metastases (Fig. 1). Staining was often patchy and heterogeneous. The mean number of cells staining in benign epithelium was 46.2%, which was lower than both cancer and lymph node metastases (79.3% and 76.4%, respectively) (Fig. 2). Each pair, benign versus cancer and cancer versus lymph node metastases, reached statistical significance (0.0016 and <0.0001, respectively). No staining was observed in urothelium, stroma, or endothelium (Fig. 1).

Adenocarcinoma showed more intense staining than benign epithelium. Lymph node metastases displayed the least intensity of staining (Fig. 3).

COMMENT

We determined the expression of PSMA in a large series of node-positive prostatic adenocarcinomas and found PSMA in 100% of cases of benign epithelium and primary cancer and 98% of lymph node metastases.

This study confirms other studies of PSMA expression with minor differences (Table I).[1,15–17] Horoszewicz *et al.*,[1] the first to describe monoclonal antibody recognition of PSMA in 1987, identified immunoreactivity in 9 of 9 prostate cancers and 2 of 2 lymph node metastases. Two other studies found PSMA immunoreactivity in the majority of primary cancers and lymph node metastases.[16,17] It is difficult to compare our study with other studies that included patients previously treated with androgen deprivation therapy or radiation.[17] PSMA expression is unchanged or increased after androgen deprivation therapy in primary cancer and lymph node metastases.[18]

PSMA expression is found in normal and malignant nonprostatic tissues.[17,19] One study reported PSMA expression in a subset of proximal renal tubules, duodenal and colonic mucosa, as well as benign and malignant prostate, and lymph node and bone metastases. This study also described intense staining in capillary endothelial cells,[17] although we did not confirm this finding in the current study or our previous report.[15] PSMA expression was also found in brain and salivary gland.[16]

PSMA, as a marker for prostatic epithelium, has many potential therapeutic applications. An immunoconjugate of 7E11-C5.3 termed CYT-356 can be radiolabeled and used to detect lymph node (greater than 5 mm) and bone metastases.[20] CYT-356 labeled with ^{111}indium (ProstaScint; Cytogen Corp.) predicts extra-prostatic cancer in pelvic lymph nodes.[13] One study described 152 patients with clinically localized cancer who were at high risk for lymph node metastases. Sixty-four patients had histologic evidence of metastases, and 40 of these were detected by the scan (sensitivity of 63%). Twenty-five patients who had positive lymph nodes on scan had lymph nodes free of cancer on histologic evaluation (false positives). At follow-up, 14 of 21 patients developed recurrent cancer.[13] Currently, there are two indications for use of ProstaScint. The first group is patients who are candidates for radical prostatectomy with high probability of metastatic cancer. Second, Prosta-

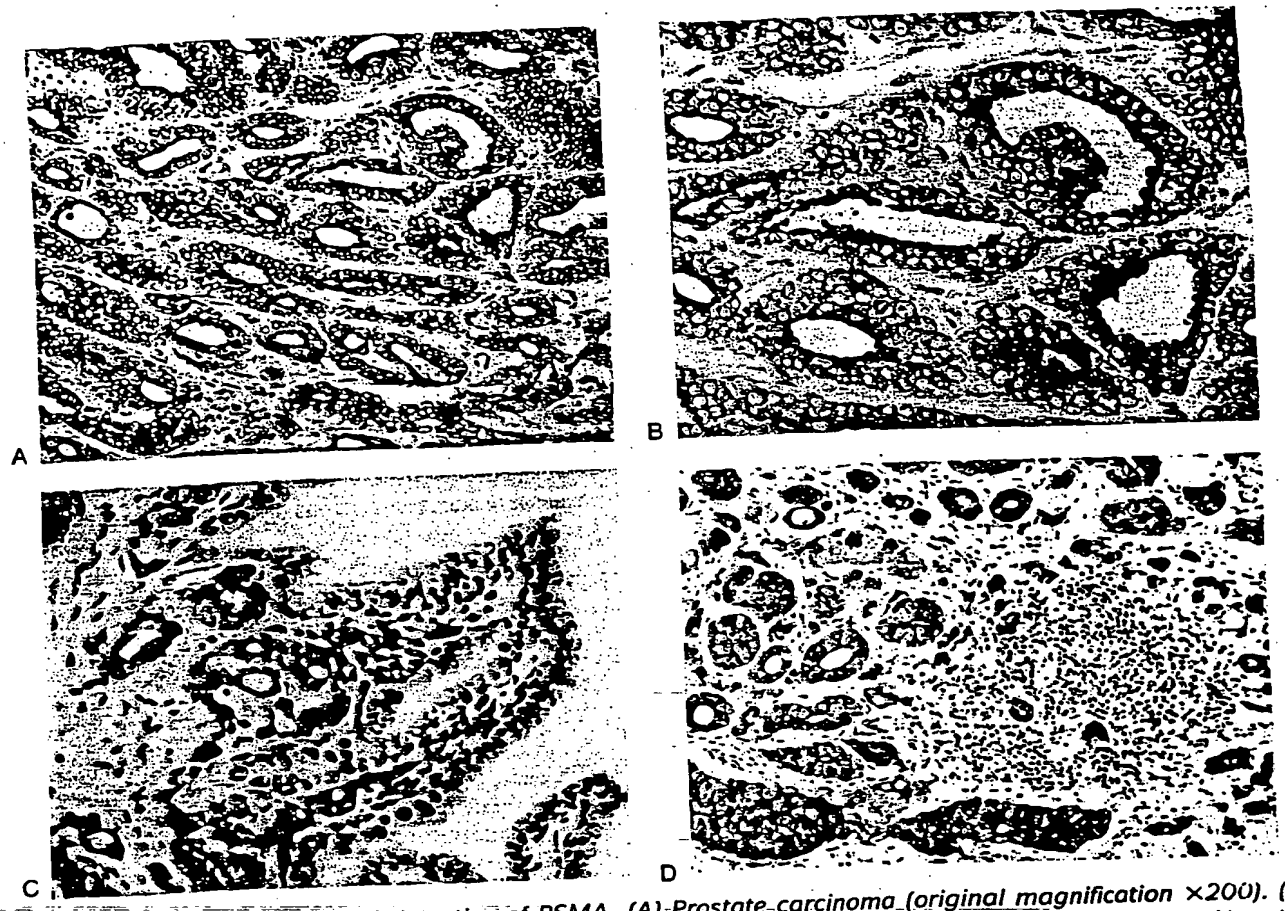

FIGURE 1. *Immunohistochemical detection of PSMA. (A) Prostate carcinoma (original magnification ×200). (B) Prostate carcinoma (original magnification ×400). (C) Invasion of prostate cancer into seminal vesicles. Note the absence of PSMA staining in the seminal vesicle epithelium. (D) Lymph node metastases.*

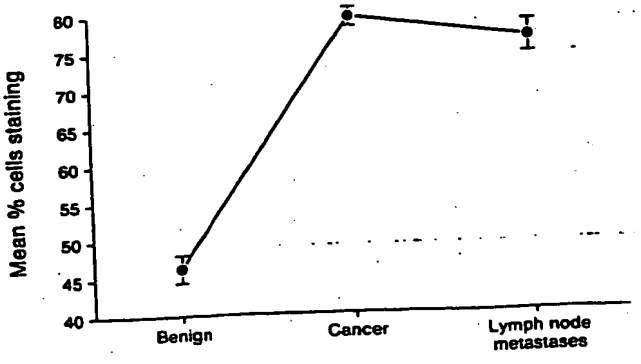

FIGURE 2. *Mean percent cells staining for PSMA.*

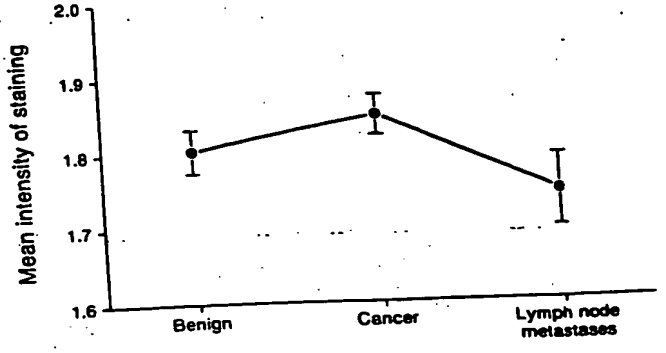

FIGURE 3. *Mean intensity of cells staining for PSMA.*

Scint is used to localize foci of prostate cancer suggested by an increasing serum prostate-specific antigen after prostatectomy.[21] Future treatments may include eradicating metastatic deposits by labeling with cytotoxic agents.[5,9]

The PSMA epitope detected by monoclonal antibody 7E11-C5 was initially thought to be intracellular and only accessible in devitalized cells undergoing apoptosis or necrosis.[13] However, a recent study reported that fluorescently labeled 7E11-C5 specifically binds to PSMA in viable prostate cancer cells,[6] providing laboratory justification for the in vivo imaging.

Serum PSMA can be detected using Western blot.[12,22–24] The normal range in healthy individuals between 35 and 55 years of age is 0.14 to 0.22 (relative intensity levels).[12] PSMA serum concentration was higher in patients with disseminated cancer (N+, M+) than in those with localized cancer (T2, T3).[23,24] PSMA concentration may be ele-

TABLE I. *PSMA expression in benign and malignant prostate*

	Benign Epithelium (%)	PIN (%)	Cancer (%)	Lymph Node Metastases (%)
Horoszewicz *et al.*[1]	71	—	100	100
Silver *et al.*[17]*	—	—	94	88
Wright *et al.*[16]	81	100	95	94
Bostwick *et al.*[15]	100	100	100	—
Current study	100	—	100	98

Key: PIN = prostatic intraepithelial neoplasia.
** Study included patients treated with androgen deprivation or radiation.*

vated after treatment, probably reflecting clinical progression or the presence of hormone-resistant cells.[1,12,24] Serum PSMA accurately predicts the stage of prostate cancer or local, regional, or distant metastases in some patients, as shown by the ProstaScint scan.[12]

In summary, we found consistent PSMA immunoreactivity in benign epithelium, primary prostate cancer, and lymph node metastases, with expression highest in cancer and lymph node metastases.

REFERENCES

1. Horoszewicz JS, Kawinski E, and Murphy GP: Monoclonal antibodies to a new antigenic marker in epithelial prostatic cells and serum of prostatic cancer patients. Anticancer Res 7: 927–936, 1987.
2. Landis SH, Murray T, Bolden S, *et al:* Cancer statistics, 1998. CA Cancer J Clin 48: 6–29, 1998.
3. Troyer JK, Beckett ML, and Wright GL Jr: Location of prostate-specific membrane antigen in the LNCaP prostate carcinoma cell line. Prostate 30: 232–242, 1997.
4. Fair WR, Israeli RS, and Heston WDW: Prostate-specific membrane antigen. Prostate 32: 140–148, 1997.
5. Israeli R, Grob M, and Fair W: Prostate-specific membrane antigen and other prostatic tumor markers on the horizon. Urol Clin North Am 24: 439–450, 1997.
6. Barren RJ III, Holmes EH, Boynton AL, *et al:* Monoclonal antibody 7E11.C5 staining of viable LNCaP cells. Prostate 30: 65–68, 1997.
7. Pinto JT, Suffoletto BP, Berzin TM, *et al:* Prostate-specific membrane antigen: a novel folate hydrolase in human prostatic carcinoma cells. Clin Cancer Res 21: 1445–1451, 1996.
8. Israeli R, Powell CT, Corr JG, *et al:* Expression of the prostate-specific membrane antigen. Cancer Res 54: 1807–1811, 1994.
9. Axelrod HR, Gilman SC, D'Aleo CJ, *et al:* Preclinical results and human immunohistochemical studies with ^{90}Y-CYT-356: a new prostatic cancer therapeutic agent (abstract). J Urol 147: 361A, 1992.
10. Gao X, Porter A, Grignon DJ, *et al:* Diagnostic and prognostic markers for human prostate cancer. Prostate 31: 264–281, 1997.
11. Babaian RJ, Sayer J, Podoloff DA, *et al:* Radioimmunoscintigraphy of pelvic lymph nodes with ^{111}indium-labeled monoclonal antibody CYT-356. J Urol 152: 1952–1955, 1994.
12. Murphy GP, Maguire RT, Rogers B, *et al:* Comparison of serum PSMA, PSA levels with results of Cytogen-356 ProstaScint scanning in prostatic cancer patients. Prostate 33: 281–285, 1997.
13. Zuckier LS, and DeNardo GL: Trials and tribulations: oncological antibody imaging comes to the fore. Semin Nucl Med 27: 10–29, 1997.
14. Bostwick DG, and Montironi R: Evaluating radical prostatectomy specimens: therapeutic and prognostic importance. Virchows Arch 430: 1–16, 1997.
15. Bostwick DG, Pacelli A, Blute M, *et al:* Prostate specific membrane antigen expression in prostatic intraepithelial neoplasia and adenocarcinoma: study of 184 cases. Cancer 82: 2256–2261, 1998.
16. Wright GL, Haley C, Beckett ML, *et al:* Expression of prostate-specific membrane antigen in normal, benign, and malignant prostate tissues. Urol Oncol 1: 18–28, 1996.
17. Silver DA, Pellicer I, Fair WR, *et al:* Prostate-specific membrane antigen expression in normal and malignant human tissues. Clin Cancer Res 3: 81–85, 1997.
18. Wright GL Jr, Grob BM, Haley C, *et al:* Upregulation of prostate-specific membrane antigen after androgen-deprivation therapy. Urology 48: 326–334, 1996.
19. Troyer JK, Beckett ML, and Wright GL Jr: Detection and characterization of the prostate-specific membrane antigen (PSMA) in tissue extracts and body fluids. Int J Cancer 62: 552–558, 1995.
20. Lopes AD, Davis WL, Rosenstraus MJ, *et al:* Immunohistochemical and pharmacokinetic characterization of the site-specific immunoconjugate CYT-356 derived from anti-prostate-monoclonal antibody 7311-C5. Cancer Res 50: 6423–6429, 1990.
21. Burgers JK, Hinkle GH, and Haseman MK: Monoclonal antibody imaging of recurrent and metastatic prostate cancer. Semin Urol 13: 103–112, 1995.
22. Rochon YP, Horoszewicz JS, Boynton AL, *et al:* Western blot assay for prostate-specific membrane antigen in serum of prostate cancer patients. Prostate 25: 219–223, 1994.
23. Murphy GP, Holmes EH, Boynton AL, *et al:* Comparison of prostate specific antigen, prostate specific membrane antigen, and LNCaP-based enzyme-linked immunosorbent assays in prostatic cancer patients and patients with benign prostatic enlargement. Prostate 26: 164–168, 1995.
24. Murphy GP, Radge H, Kenny GM, *et al:* Comparison of prostate specific membrane antigen, and prostate specific antigen levels in prostatic cancer patients. Anticancer Res 15: 1473–1480, 1995.